Vitamin D: A Micronutrient Regulating Genes

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Abstract: Background: At sufficient sun exposure, humans can synthesize vitamin D₃ endogenously in their skin, but today's lifestyle makes the secosteroid a true vitamin that needs to be taken up by diet or supplementation with pills. The vitamin D₃ metabolite 1α,25-dihydroxyvitamin D₃ acts as a nuclear hormone activating the transcription factor vitamin D receptor (VDR).

Methods: This review discusses the biological effects of micronutrient vitamin D ranging from calcium homeostasis and bone formation to the modulation of innate and adaptive immunity.

Results: Since normal human diet is sufficient in vitamin D, the need for efficient vitamin D₃ synthesis in the skin acts as an evolutionary driver for its lightening during the migration out of Africa towards North. Via activating the VDR, vitamin D has direct effects on the epigenome and the expression of more than 1000 genes in most human tissues and cell types.

Conclusions: The pleiotropic action of vitamin D in health and disease prevention is explained through complex gene regulatory events of the transcription factor VDR.

Keywords: Vitamin D, VDR, vitamin D response index, vitamin D supplementation, epigenome, transcriptome, gene regulation, evolution.

1. INTRODUCTION

In an evolutionary perspective, the synthesis of cholesterol is an old biochemical pathway, which was used already by simple organisms, such as phyto- and zooplankton [1, 2]. For this reason, for more than 500 million years, all cholesterol synthesizing species contain rather abundant amounts of the cholesterol precursor 7-dehydrocholesterol (also called pro-vitamin D₃, Fig. 1). When the latter molecule is close to the body's surface and exposed to UV-B (290-315 nm), the thermodynamically unstable molecule pre-vitamin D₃ is produced in a non-enzymatic reaction using the energy by the UV radiation (most efficient are 295-297 nm). Pre-vitamin D₃ then further isomerizes into vitamin D₃ (also called cholecalciferol, Fig. 1).

Phyto- and zooplankton use vitamin D synthesis as a chemical sunscreen but not as an endocrine compound [3]. In this way, vitamin D₂ and vitamin D₃ accumulate in the marine food chain and explain why the liver of fatty fish has rather high concentrations of the molecule. Since some 100 years, it is known that cod-liver oil contains a rather high concentration of a molecule that was termed "vitamin D", because it was the vitamin to be named fourth in line [4]. Thus, cod-liver oil could rickettes, a childhood disease of bone malformation, by providing supplementation with vitamin D₃ [5]. Even earlier, sunlight exposure was used as an efficient therapy of rickettes as well as of tuberculosis, which is an infectious disease caused by intracellular bacteria. Since humans can synthesize vitamin D₃ in their skin, the sunshine cure led to endogenous production in the patients, to overcomethe vitamin D deficiency [6].

The ability of humans to synthesize vitamin D₃ in their skin suggests that the term "vitamin" may not be correctly used. However, during the last thousands of years, humans tend to stay preferentially indoors and cover their skin by textile outdoors leading to insufficient UV-B exposure [7]. The resulting low endogenous vitamin D₃ production makes the compound a vitamin, i.e. an essential micronutrient, for a huge proportion of the human population.

Although some mushrooms and plants, when exposed to UV-B, can produce a vitamin D isomer (vitamin D₂/ergocalciferol), average human diet does not contain much vitamin D. Therefore, some countries apply the fortification of dietary products, such as milk, margarine and juices, with vitamin D₃ (or vitamin D₂) and/or recommend direct vitamin D supplementation via pills [8].

This review will discuss the role of the micronutrient vitamin D₃ as a signaling molecule that mediates its physiological actions via its nuclear receptor VDR, that has effects on the epigenome and gene expression.

2. VITAMIN D METABOLITES

The molecule vitamin D₃ is biologically inert, but hydroxylation at carbon 25 of its side chain results in 25-hydroxyvitamin D₃ (25(OH)D₃, also called calcidiol, Fig. 1), which is the most abundant form of vitamin D displaying a serum half-life of 15 days. Therefore, serum 25(OH)D₃ levels are traditionally used as a biomarker for the vitamin D status [9]. Furthermore, a hydroxylation at carbon 1 within the A-ring of 25(OH)D₃ creates the biologically most active vitamin D metabolite, 1α,25-dihydroxyvitamin D₃ (1,25(OH)₂D₃, also called calcitriol, Fig. 1). Interestingly, some vitamin D metabolites, such as 20-hydroxy- and 20,23-dihydroxyvitamin D₃, seem to act as antagonists for the nuclear receptors RORα and RARY [10].

1,25(OH)₂D₃ is an endocrine hormone and the main source of its production are proximal tubule cells of the kidneys. In addition, also monocytes, macrophages and dendritic cells of the innate immune system, osteoblasts within bones and keratinocytes of the skin can produce 1,25(OH)₂D₃. The high-affinity receptor (Kd 0.1 nM) of 1,25(OH)₂D₃, VDR, is a member of the nuclear receptor superfamily and via this transcription factor, vitamin D has a direct effect on gene regulation (more details below) [11].

The degradation of 25(OH)D₃ and 1,25(OH)₂D₃ is initiated by hydroxylation at carbon 24 (Fig. 1). Interestingly, the CYP24A1 gene, which encodes for the 24-hydroxylase responsible for vitamin D degradation, is one of the most induced primary VDR targets. In contrast, the CYP27B1 gene encoding for 1α-hydroxylase responsible for 1,25(OH)₂D₃ production is down-regulated by VDR and its
Vitamin D became an endocrine compound, when species left the ocean and had to develop a stable calcium-based skeleton [13]. Thus, only in vertebrates, a full vitamin D endocrine system composed of a high-affinity receptor (VDR), a transporter (vitamin D binding protein) and metabolizing enzymes (the cytochrome P450 enzymes CYP2R1, CYP27B1 and CYP24A1) is found (Fig. 1). Vitamin D endocrinology may have focused first on the control of calcium homeostasis [13] but later was also observed to be involved in non-skeletal functions, such as modulating cellular growth and differentiation as well as the immune system.

The whole body’s calcium metabolism is coordinated by the intestine, kidney, bone, fat and brain, in order to keep serum calcium levels in a narrow range [14]. The main role of vitamin D in this physiologically important process is the stimulation of the absorption of calcium and phosphorus from food within the intestine as well as the support of the re-absorption of calcium in renal tubules [15]. Key vitamin D-regulated genes in this process are a calcium channel in enterocytes (encoded by the gene TRPV6) as well as calbindin 1 and 2 (encoded by the genes CALB1 and CALB2) that transport calcium in the intestine. Furthermore, fibroblast growth factor 23 (encoded by the vitamin D target gene FGF23) regulates phosphate homeostasis and transport in the kidneys [16]. Thus, sufficient levels of vitamin D ensure calcium and phosphorus homeostasis and support in this way bone remodeling. There seems to be no direct effects of vitamin D on bone mineralization, but the differentiation of monocytes into bone-resorbing osteoclasts is under the control of the cytokine RANKL, which is encoded by the vitamin D target gene TNFSF11 [17].

Bone malformations, such as rickets, are the dominant phenotypic rare occurrence of humans carrying mutations in their FDR gene [18]. Thus, these natural VDR knockout cases indicate that calcium homeostasis and its effects on bone health are the most prominent functions of vitamin D. However, the rather ubiquitous expression of VDR (discussed below) suggests that vitamin D is also involved in a number of non-skeletal functions, of which the regulation of cellular differentiation and growth [19] and the control of innate and adaptive immunity [20] have been studied most intensively.

Humans lost their body hair approximately 1 million years ago, and increase their physical performance via improved sweating-mediated body temperature control. In parallel, these early humans increased melanin production in their skin, i.e., got dark skin, in order to protect their circulating folate (an important methyl-group donor, e.g., for DNA methylation) from UV degradation in sunny East Africa. Despite an efficient melanin sun shield (dark-skinned Africans need about 5 to 6 times more UV-B light than fair-skinned individuals), the intensive sun at the equator allows sufficient vitamin D3 synthesis. Accordingly, traditionally living Massai people in Tanzania and Kenya show an average 25(OH)D3 serum level of 119 nM (ranging from 58 to 167 nM [21]). More than 200,000 years ago, anatomically modern humans developed and about 50,000 years ago some of them moved towards Asia and Europe. The need for vitamin D3 production in the skin despite less intense sun exposure in these geographic regions created a strong evolutionary pressure for skin lightening [22]. The latter process took 10,000 to 30,000 years, which is, on an evolutionary scale, very fast. Thus, light skin color is the result of an evolutionary adaptation process, in order to keep vitamin D3 synthesis reasonably high.

In the context of agricultural revolution, some 10,000 years ago, the lifestyle of nearly all human populations started to change. Even more drastic lifestyle changes happened during the industrial revolution within the past 200 years. This affected the composition of diet and of the intestinal microbiome as well as physical activity. However, most genetic adaptations take far longer, so that most today’s humans are still primarily genetically adapted to the environmental conditions and lifestyle of their ancestors in East Africa, i.e., the human body is still primarily adapted to a constant vitamin D status [23]. While some nuclear hormones, such as the steroid hormones cortisol and estradiol, show daily or monthly fluctuations, respectively, the levels of 1,25(OH)2D3 are supposed to stay rather constant over time. However, major seasonal changes in sun
exposure, such as occurring at a latitude above 37 °N, can cause, in the absence of supplementation via diet or pills, variations in the vitamin D status and deficiency during winter months.

These changes in the vitamin D status cause homeostatic imbalances that may contribute to a number of non-communicative disorders. Interestingly, vitamin D deficiency does not only result in bone-related problems, such as rickets in children or a higher risk for fractures in adults [24], but also compromises the protective roles of vitamin D for diseases like cancer, diabetes, cardiovascular diseases, neuropsychiatric disorders and infections [25]. A prominent example is the autoimmune disease multiple sclerosis, which is suggested to be largely preventable by a sufficient vitamin D status of the persons at risk [26].

4. VITAMIN D SUPPLEMENTATION

For bone good health, such as the prevention of fractures caused by osteoporosis, it is important to keep a sufficient vitamin D status [27]. The latter is classified via 25(OH)D3 serum concentrations as i) severely deficient (< 12.5 nM), ii) deficient (12.5-25 nM), iii) insufficient (25-50 nM) and iv) sufficient (> 50 nM) [28]. Accordingly, for both bone and overall health, the US Institute of Medicine recommends 25(OH)D3 serum levels of > 50 nM [27]. This should be reached by a daily supplementation with 10-15 µg (400-600 IU) vitamin D3 for children and with 15-20 µg (600-800 IU) for adults. In contrast, the US Endocrine Society recommends that the 25(OH)D3 serum level of humans should be at least 75 nM, i.e. daily supplementations with 25 µg (1000 IU) vitamin D3 or more [29]. Too high oral vitamin D3 intakes can have toxic effects, such as hypercalcemia, which is caused by increased intestinal calcium absorption and mobilization of calcium from bone and can result in soft tissue calcification [30]. Therefore, the upper limit for daily vitamin D3 supplementation is 100 µg (4000 IU).

Analysis of vitamin D3 supplementation studies, such as VitDmet (NCT01479933) [31-34] and VitDbol (NCT02063334) [35, 36], suggested that individuals show a personal molecular response to supplementation with vitamin D3, which is independent of their vitamin D status. The molecular response to vitamin D3 supplementation is expressed by the vitamin D response index and classifies humans into high, mid or low responders to vitamin D [7, 37]. Accordingly, there should be rather a personalized vitamin D3 supplementation than a general, population-based recommendation, in order to reach the individual's optimal vitamin D status. Thus, the vitamin D response index concept may dissolve the scientific dispute which 25(OH)D3 serum levels should be targeted and which vitamin D3 amounts should be recommended for daily supplementation. In addition, a use of the vitamin D response index for the stratification of vitamin D intervention studies cohorts may help in proving or disproving observations that a high vitamin D status protects against cardiovascular disease, diabetes, colorectal cancer and all-cause mortality.

5. CENTRAL ROLE OF THE VDR

The biologically most active form of vitamin D, 1,25(OH)2D3, is a lipophilic molecule that easily passes through biological membranes and activates in the nucleus its receptor VDR (Fig. 2). Thus, signal transduction by vitamin D is more direct than that of hydrophilic signaling molecules, such as peptide hormones, growth factors and cytokines, that cannot enter the cell and have to activate a membrane receptor, in order to mediate the effects. However, non-genomic and rapid actions of 1,25(OH)2D3 at the cellular membrane and in the cytosol, such as a rapid calcium transport mechanism termed transcalctachia, have been described in the intestine, vascular smooth muscle and pancreatic β-cells [38]. Occasionally, VDR is found in the cytoplasm and was shown to interact with membrane invaginations (caveolae). In these cases, vitamin D stimulates signal transduction pathways that are mediated by mitogen-activated protein kinase (MAPK) and cyclic adenosine monophosphate. Similarly, 1,25(OH)2D3 induces a transcription-independent Ca2+ influx and the activation of the kinases MAPK14 and RPS6KA5 [39]. Nevertheless, the general belief is that the vast majority of the physiological actions of vitamin D are mediated by VDR’s genomic actions in the nucleus [40].

A transcription factor, such as VDR, contacts genomic DNA in a sequence-specific fashion, i.e. it binds to a genomic region only when its preferred sequence motifs are accessible. There are approximately 1600 human transcription factors, a few of which are expressed ubiquitously but most are specific to a limited number of tissues and cell types [41]. VDR protein occurs preferentially in intestine and kidneys as well as in skin, parathyroid gland and pituitary gland (www.proteinatlas.org/ENSG00000111424-VDR/tissue), but lower expression levels are also found in most of the 400 other human tissues and cell types. In general, transcription factors do not need a high level expression for their effective function, suggesting that most human tissues are sensitive to vitamin D.

Like other members of the nuclear receptor superfamily, VDR carries a structurally conserved ligand-binding domain [11]. The inner surface (some 40 amino acids) of the latter domain forms a relatively small ligand-binding pocket that snugly encloses 1,25(OH)2D3 and binds it with high affinity [42]. In turn, the outer surface of the ligand-binding domain is the main interface for VDR’s interaction with other nuclear proteins, such as members of the NCOA family of histone acetyltransferases [43] and of the co-repressor proteins NCOR1 mediating the contact with histone deacetylases [44]. Moreover, VDR communicates with chromatin modifying proteins, such as the lysine demethylase KDM6B [45] and the chromatin remodeler BRD7. These proteins are either found together with VDR within the same large protein complex [46, 47] or their genes are targets of vitamin D [48].

VDR’s DNA-binding domain recognizes the hexameric sequence A/GGG/TTC/CA. Heterodimeric complexes of VDR with the retinoid X receptor preferentially bind to a direct repeat of these hexameric motifs spaced by three nucleotides, so-called DR3-type response elements [49]. In fact, the method chromatin immunoprecipitation combined with massive parallel sequencing (ChIP-seq) demonstrated on a genome-wide level that DR3-type sequences are the most prominent binding motifs below the summits of VDR peaks [50-54].

The VDR cistrome, i.e. the genome-wide VDR binding pattern [55], comprises some 5-20,000 sites per cell type. In human it had been described for B lymphocytes [50], monocytes [51, 56], colorectal cancer cells [53], hepatic stellate cells [54] and macrophage-like cells [52]. In all these in vitro cell culture models, vitamin D stimulation clearly increases the number of genomic VDR binding events [52]. Nevertheless, the VDR cistrome is rather cell-specific [52] explaining why most tissues and cell types that express VDR have rather different vitamin D target genes [55, 57].

6. EPIGENOME-WIDE EFFECTS OF VITAMIN D

Epigenetics was initially understood as the genetic process behind development and cellular differentiation [58], but it also comprises all functionally relevant changes of the genome that do not involve any alteration in the nucleotide sequence but are still potentially heritable [59]. The complex of genomic DNA with nucleosomes is called chromatin and has an intrinsic repressive potential, in order to stabilize the epigenetic landscape of a differentiated cell [60]. This implies that all changes in chromatin represent changes of the epigenome. For the four different histone proteins that form a nucleosome more than 100 post-translational modifications, such acetylations and methylations, are known [61]. These histone modifications affect the non-covalent interactions within and between nucleosomes and alter the structure of chromatin. Moreover, changes in DNA methylation, preferentially within so-called " CpG islands", largely contribute to the epigenome [62]. Finally, also higher order chromatin structures, such organization of
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1. Epigenome → 2. Transcriptome

Fig. (2). Gene regulation by vitamin D. VDR can binds to some 10,000 sites within the genome, if they are located within accessible chromatin. 1,25(OH)2D3-activated VDR mediates via its nuclear interaction partner proteins the opening and closing of chromatin, i.e. the primary effect of vitamin D stimulations are changes of the epigenome. Some of these epigenome-wide effects translate into the changes of the transcriptome.

The VDR cistrome contains a few hundred persistent loci, the binding to which changes significantly over time after ligand stimulation, but which always stay occupied [65]. A gene can only be transcribed when the genomic regions of both its transcription start site (TSS) and the binding sites of the transcription factors controlling the activity of RNA polymerase II, referred to as enhancers, are located within accessible chromatin [66]. Prominent markers of the latter are H3K27ac modifications at enhancer regions and H3K4me3 at TSS regions. Both types of histone markers were found to be modulated by vitamin D [67]. By default, chromatin largely restricts the access of transcription factors to promoter and enhancer regions, so that per cell type only a small number of sites within accessible chromatin already in the absence of ligand, but 1,25(OH)2D3 stimulation increases the number of DNA-bound VDR molecules supported of pioneer factors like as PU.1 (Fig. 2). This leads to changes in chromatin accessibility and changes in histone marks, which in turn increases the binding strength of CTCF sites that form TAD anchors upstream and downstream of prominent VDR binding sites [76].

In the chromatin model of vitamin D signaling, VDR binds to a small number of sites within accessible chromatin already in the absence of ligand, but 1,25(OH)2D3 stimulation increases the number of DNA-bound VDR molecules supported of pioneer factors like as PU.1 (Fig. 2). This leads to changes in chromatin accessibility and changes in histone marks, which in turn increases the binding strength of CTCF sites that form TAD anchors upstream and downstream of prominent VDR binding sites [76].

To date, most genome-wide data are available for the actions of vitamin D in the cells of the hematopoietic system [55]. This emphasizes the impact of VDR and vitamin D for innate and adaptive immunity. For example, the transcriptome of THP-1 human monocytes is affected by 1,25(OH)2D3 at more than 500 genes [73].

CONCLUSION

The micronutrient vitamin D has pleiotropic physiological functions, since it not only regulates calcium and phosphorus homeostasis but also modulates innate and adaptive immune responses [19,
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